Intramyocardial Bone Marrow Cell Injection: Clinical and Functional Effects in Ischemic Heart Disease

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Chapter 2

Intramyocardial Bone Marrow Cell Injection for Chronic Myocardial Ischemia: A Randomized, Double-blind, Placebo-controlled Trial

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Journal of the American Medical Association *2009;* 301:1997-2004

ABSTRACT

Context: Previous studies suggested that bone marrow cell injection may improve myocardial perfusion and left ventricular (LV) function in patients with chronic myocardial ischemia.

Objective: To investigate the effect of intramyocardial bone marrow cell injection on myocardial perfusion and LV function in patients with chronic myocardial ischemia.

Design, Setting and Patients: A randomized, double-blind, placebo-controlled trial at a university hospital (Leiden, the Netherlands). Between May 1st 2005 and March 3rd 2008, 50 patients with chronic myocardial ischemia (64±8 years, 43 male) were enrolled. The inclusion criteria were severe angina pectoris despite optimal medical therapy and myocardial ischemia on Tc-99m tetrofosmin single-photon emission computed tomography (SPECT). All patients were ineligible for conventional revascularization. The final 6 months follow-up was completed in September 2008.

Interventions: Intramyocardial injection of $100x10^6$ autologous bone marrow–derived mononuclear cells or placebo solution.

Main Outcome Measure: The primary outcome measure was the summed stress score, which is a 17-segment score for stress myocardial perfusion as assessed by SPECT.. Secondary outcome measures included LV ejection fraction, Canadian Cardiovascular Society (CCS) class (range I-IV) and quality-of-life as measured by the Seattle Angina Questionnaire (range 0-100%, with a mean difference of more than 5% considered clinically significant).

Results: After 3 months follow-up, the summed stress score improved from 23.5±4.7 to 20.1±4.6 (P<0.001) in the bone marrow cell group, compared to a decrease from 24.8±5.5 to 23.7±5.4 (P<0.004) in the placebo group (bone marrow cell treatment effect -2.44, 95% CI, -3.58, -1.30, P<0.001). In the bone marrow cell-treated patients who underwent magnetic resonance imaging (MRI), a 3% absolute increase in LV ejection fraction was observed at 3 months (95% CI 0.5, 4.7, n=18). In the placebo group, MRI showed no improvement in LV ejection fraction (absolute difference -1%, 95% CI -2.1, 1.1, n=22, P=0.027 between

groups). CCS angina score improved significantly in the bone marrow cell group (absolute difference at 6 months, -0.79, 95% CI -1.10, -0.48, P<0.001) compared with no significant improvement in the placebo group (absolute difference at 6 months -0.39, 95% CI -0.9, 0.12, P=0.058). Quality-of-life increased from 56±9% to 64±12% at 3 months and 69±12% at 6 months in bone marrow cell-treated patients (absolute improvement at 6 months 13.0%, 95% CI 8.2, 17.9, P<0.001), compared to a smaller increase in the placebo group (57±11% vs. 61±14% vs. 64±17%, absolute improvement at 6 months 6.3%, 95% CI 0.5, 13.0, P=0.036). The improvements in CCS class and quality-of-life were significantly greater in bone marrow cell-treated patients than in placebo-treated patients (P=0.032 and P=0.037 respectively).

Conclusion: In this short-term study of patients with chronic myocardial ischemia refractory to medical treatment, intramyocardial bone marrow cell injection resulted in a statistically significant but modest improvement in myocardial perfusion compared to placebo. Further studies are required to assess long-term results and the efficacy for mortality and morbidity.

Trial Registration: trialregister.nl identifier: NTR400

INTRODUCTION

Bone marrow cell therapy is currently being investigated as a new therapeutic option for patients with ischemic heart disease. This treatment aims to improve myocardial perfusion and contractile performance through administration of therapeutic cells into ischemically-damaged myocardium. The majority of clinical studies conducted so far investigated whether intracoronary bone marrow cell infusion can enhance functional recovery after acute myocardial infarction.[1-3]

Animal model studies, however, suggested that bone marrow cell therapy may also improve myocardial perfusion and increase left ventricular (LV) function in chronic ischemia.[4,5] A number of non-randomized clinical studies indicated the safety and feasibility of intramyocardial bone marrow cell injection. Moreover, a beneficial effect on myocardial perfusion and LV function was presumed. Until now, only 2 small-sized randomized controlled studies assessed the effect of bone marrow cell injection in patients with chronic myocardial ischemia.[6,7] Since the results of these 2 studies were

discrepant, the beneficial effect of this treatment modality on myocardial perfusion and LV function remains unclear.
The aim of the current randomized, double-blind, placebo-controlled trial was to assess the effect of intramyocardial bone marrow cell injection on myocardial perfusion and LV function in patients with chronic ischemia who are ineligible for conventional treatment.

METHODS

Patients

The study population consisted of patients with severe angina (Canadian Cardiovascular Society (CCS) class III-IV) despite optimal medical therapy and myocardial ischemia in at least 1 myocardial segment on Tc-99m tetrofosmin single-photon emission computed tomography (SPECT). All patients were ineligible for both surgical and percutaneous revascularization, as determined by an independent expert panel that reviewed the coronary angiograms.
Exclusion criteria were: LV ejection fraction (LVEF) <35%, acute myocardial infarction within 6 months before enrollment, history of malignancy, renal dysfunction (glomerular filtration rate <30 ml/min/1,73m^2)), or unexplained hematological abnormalities. The protocol was approved by the institutional ethics committee and written informed consent was obtained from each patient. The study was registered at the Dutch trial registry (www.trialregister.nl, no. NTR400/ISRCTN58194927).

Study protocol

At baseline, the clinical status of the patients was assessed according to the CCS classification, ranging from class I (mild) to IV (severe)[8]. Quality-of-life was assessed using the disease specific Seattle Angina Questionnaire. This questionnaire results in a score ranging from 0 to 100,% with the higher scores indicating better health status. A mean difference of more than 5% was considered clinically significant.[9,10]. A bicycle exercise test (to evaluate exercise capacity), SPECT (to assess myocardial perfusion) and magnetic resonance imaging (MRI, to assess LV function and volumes) were performed at baseline.
On the day of the injection procedure, 80 ml bone marrow was harvested from each patient. The bone marrow was aspirated from the iliac crest by an experienced hematologist under local anesthesia and placed in heparinised Hanks balanced salt solution. Mononuclear

cells were isolated as previously described using Ficoll density gradient centrifugation according to Good Manufacturing Practice regulations, washed in phosphate buffered saline with 0.5% human serum albumin and resuspended in phosphate buffered saline with 0.5% human serum albumin.[11] The final suspension contained $40x10^6$ bone marrow mononuclear cells/ml. The filtered bone marrow was checked for the presence of clots and the bone marrow cell population was analyzed by fluorescence-activated cell sorting using anti-CD34 and anti-CD45 antibodies (Becton Dickinson, Palo Alto, California).

After cell isolation, patients were randomly assigned in a 1:1 ratio to the bone marrow cell group or the placebo group, using sequentially numbered sealed envelopes provided by the Department of Medical Statistics and Bioinformatics. A block size of 4 was used without further stratification. Following randomization, a blinded syringe with either bone marrow cell suspension or placebo solution (NaCl 0.9% with 0.5% human serum albumin) was brought to the catheterization laboratory. Thus, the patients, the study coordinators and the investigators who were responsible for patient assessment were unaware of group assignment.

During cell isolation and randomization, a 3D-electromechanical map of the LV was obtained as previously reported[12] using the NOGA system (BDS, Cordis, California, USA). The ischemic regions on SPECT were visually matched with the 3D-electromechanical map, based on anatomical landmarks including LV long axis, position of apex, mitral valve area, aortic valve location and basal inferoseptal point. Cross-referencing was also performed using fluoroscopic identification of anterior, septal, lateral and inferior orientations. Subsequently, 8-10 intramyocardial injections of 0.2-0.3 ml each were delivered.

At 3 and 6 months, CCS class, quality-of-life and exercise capacity were re-assessed and 24-hour Holter electrocardiogram recordings were obtained to monitor the occurrence of arrhythmias. SPECT and MRI were repeated at 3 months to re-assess myocardial perfusion and LV function and volumes.

SPECT imaging

SPECT imaging was performed as previously described.[12] Briefly, adenosine stress was used (0.14 mg/kg/min for 6 minutes), and 500 MBq Tc-99m tetrofosmin was injected intravenously after 3.5 minutes of adenosine infusion. Resting images were obtained after a second injection of 500 MBq Tc-99m tetrofosmin. Reconstruction yielded long-

and short-axis projections perpendicular to the heart axis. The short-axis slices were displayed in polar map format, adjusted for peak myocardial activity (100%).
The myocardium was divided into 17 segments according to the AHA/ACC recommendations.[13] Myocardial perfusion was analyzed quantitatively (QGS-software) and segmental tracer activity was categorized on a 4-point scale: 1=tracer activity >75%; 2=tracer activity 50-75%; 3=tracer activity 25-50%; 4=tracer activity <25%. Perfusion defects on stress images were considered present when tracer activity was <75% of maximum. When significant fill-in (>10%) of perfusion defects was observed on the resting images, segments were classified as ischemic. Summation of the patients' segmental scores at stress yielded the summed stress score and summation of the patients' segmental rest scores yielded the summed rest score.

MRI

MRI studies were performed as previously described.[11] A 1.5-Tesla system (Philips Medical Systems, Best, The Netherlands) with 5-segment synergy coil and vector electrocardiographic gating was used. Two experienced observers (J.V.R and S.D.R), blinded to all clinical data, analyzed the images. Previously validated software was used to determine parameters of global systolic function (MASS, Medis, the Netherlands).[14] As reported, the intra- and interobserver variability were 1±3 ml and 2±4 ml for LV end-systolic volume (LVESV), 1±4 ml and 2±6 ml for LV end-diastolic volume (LVEDV), and 0.2±1.6% and 0.5±2.1% for LVEF.[11]

Assessment of exercise capacity

Patients performed a symptom-limited bicycle exercise test with a 20 Watt starting load and 10 Watt increment per minute. Exercise tests were monitored by a study coordinator who was not aware of the patients' group assignment. Patients were encouraged to perform as much exercise as possible, while their symptoms and 12-lead electrocardiogram (ECG) were continuously assessed. Test endpoints were angina, physical exhaustion, dyspnea or significant decrease in blood pressure (>10 mmHg).

Statistical analysis

This study was designed to demonstrate a treatment difference of at least 3 points in summed stress score between bone marrow cell-treated patients and placebo-treated patients. Based on an earlier study from our group, the standard deviation (SD) of this

effect measure was estimated at 3.35 points.[12] Then, to obtain a power of at least 85% in a two-sided test with a type I error of at most 5%, 23 patients needed to be enrolled in each group. Fifty patients were enrolled to account for dropouts.

Data are reported as mean±SD. Categorical variables were compared using the chi-square test or Fischer's exact test. We applied repeated measures analysis of variance (ANOVA), as well as the Friedman test, to study the relation between randomly allocated treatment and (changes in) continuous (outcome) data at baseline and follow-up time points. For the analysis of summed stress score, summed rest score and variables with significant differences in baseline values, an analysis of covariance (ANCOVA) analysis was performed to assess differences between both groups at 3 months follow-up, adjusted for baseline values. The ANCOVA included the values at 3 months as dependent variables and the associated baseline values and a factor for treatment as independent variables. Treatment effects were estimated by computing the differences between the adjusted means of the bone marrow cell-treated patients and placebo-treated patients and their corresponding 95% confidence intervals (95% CI). A Mann-Whitney test was used to compare changes in semi-quantitative data. A P-value <0.05 was considered significant. All statistical analyses were performed with SPSS software (version 16.0, SPSS).

Funding

The present study is an academia-initiated exploratory phase II study. No external sponsor was involved in study design, data collection, data analysis, data interpretation, or writing of the report.

RESULTS

Between May 1st 2005 and March 3rd 2008, 50 patients (64±8 years, 43 male) were enrolled in the study. Of these, 25 were assigned to the bone marrow cell group, and 25 to the placebo group. All analyses were performed in line with the intention-to-treat principle. For all paired tests, complete case analysis was performed. There were no differences in baseline characteristics between the groups (Table 1). During the study period, the type and dose of cardiovascular medications remained unchanged in all patients.

Table 1. Baseline characteristics of the study population

	Bone marrow cell group (n=25)		Placebo group (n=25)		P-value
Age, years	64±8		62±9		0.55
Gender (Male)	23	(92%)	20	(80%)	0.41
Cardiovascular risk factors					
Smoking	10	(40%)	12	(48%)	0.77
Hypertension	12	(48%)	11	(44%)	1.00
Diabetes	13	(52%)	8	(32%)	0.25
Dyslipidemia	12	(48%)	15	(60%)	0.57
Family history of CAD	16	(64%)	13	(52%)	0.56
BMI	29±3		28±4		0.40
Current Medication					
Nitrates	21	(84%)	21	(84%)	1.00
Beta-blockers	24	(96%)	24	(96%)	1.00
Calcium channel blockers	18	(72%)	18	(72%)	1.00
Statins	25	(100%)	25	(100%)	1.00
ACE inhibitors	19	(76%)	14	(56%)	0.23
Clopidogrel	13	(52%)	8	(32%)	0.25
Aspirin	20	(80%)	21	(84%)	1.00
Oral anticoagulants	5	(20%)	4	(16%)	0.68
History					
Prior Myocardial infarction	14	(56%)	18	(72%)	0.37
Prior CABG	24	(96%)	19	(76%)	0.10
Prior PCI	16	(64%)	13	(52%)	0.57

CAD = coronary artery disease; BMI = body mass index; ACE = angiotensin converting enzyme; CABG = coronary artery bypass grafting; PCI = percutaneous coronary intervention.

Procedural data

Mean procedural time for bone marrow aspiration was 20±8 minutes in the bone marrow cell group and 23±9 minutes in the placebo group (P=0.22). Procedural time for mapping and injection was 62±18 minutes in the bone marrow cell group and 59±16 minutes in the placebo group (P=0.42). Bone marrow cell-treated patients received 8.5±1.3 injections, whereas placebo-treated patients received 8.3±1.0 injections (P=0.24). The cell suspension contained 98±6x10^6 bone marrow cells, with a cell viability of 98±1% and a CD34-positive cell fraction of 2.4±0.9%.

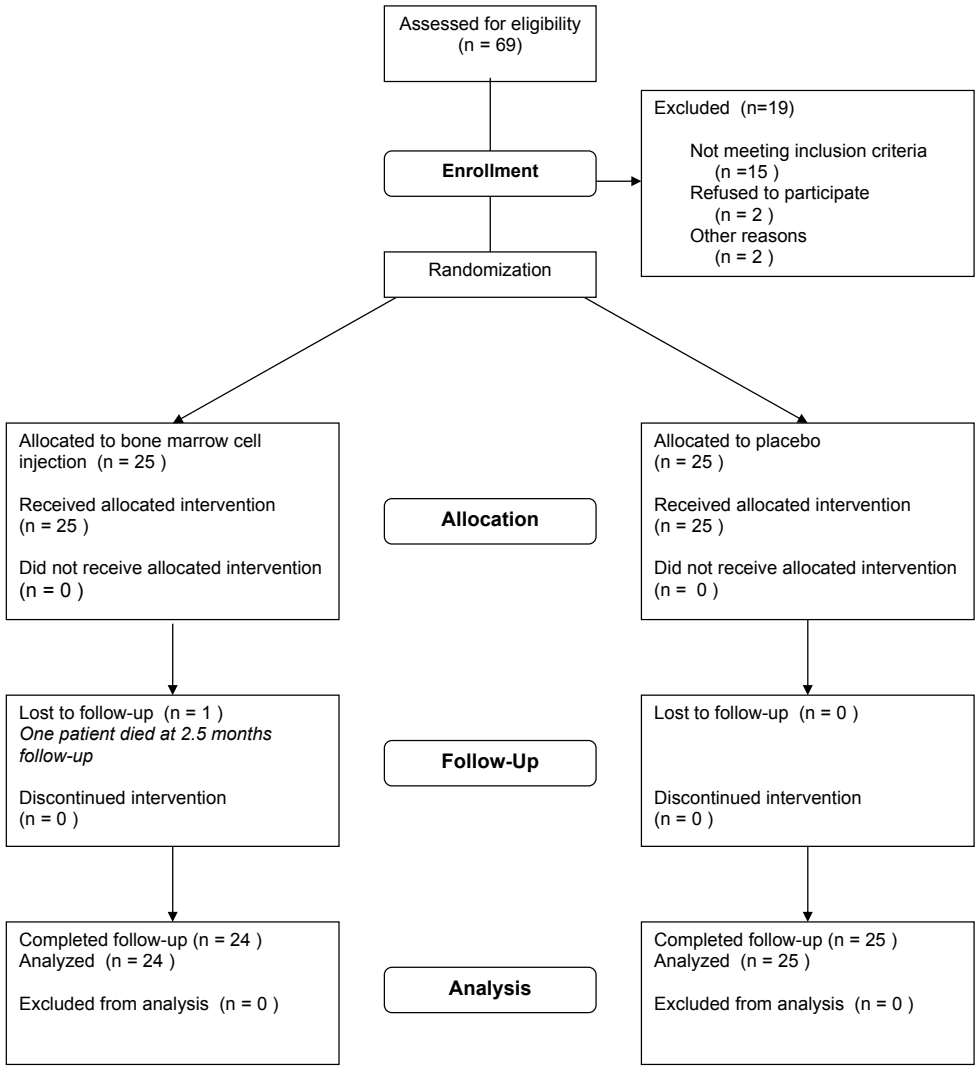

Figure 1 Flow of participants through the trial.

Myocardial perfusion and ischemia

Paired SPECT studies were available for 24 bone marrow cell-treated patients and 25 placebo-treated patients. In the bone marrow cell group, the summed stress score improved from 23.5±4.7 at baseline to 20.1±4.6 at 3 months ($P<0.001$). In the placebo group, the summed stress score also improved (from 24.8±5.5 at baseline to 23.7±5.4 at 3 months, $P=0.004$). However, when the 2 groups were compared, the improvement

in summed stress score was significantly greater in bone marrow cell-treated patients as compared to placebo-treated patients (treatment effect -2.44, 95% CI, -3.58, -1.30, P<0.001). The summed rest score improved from 18.9±3.9 to 18.3±3.9 at 3 months in the bone marrow cell group (P=0.002). In the placebo group, the summed rest score remained unchanged (21.0±5.4 vs. 20.7±4.8 at 3 months, P=0.10). The change in summed rest score was not significantly different between both groups (treatment effect -0.32, 95% CI -0.87, 0.23, P=0.25).

The number of ischemic myocardial segments per patient at baseline was not significantly different between the groups (P=0.10). In the bone marrow cell group, the mean number of ischemic myocardial segments per patient decreased from 3.9±1.8 at baseline to 1.5±1.5 at 3 months (P<0.001). In the placebo group, the number of ischemic myocardial segments also decreased from 3.1±1.5 baseline to 2.4±1.8 at 3 months (P=0.003). Figure 2 displays that the absolute decrease in the number of ischemic myocardial segments per patient was significantly greater in the bone marrow cell group (-2.4, 95% CI -2.9, -1.9 vs. -0.8, 95% CI -1.2, -0.3, P<0.001).

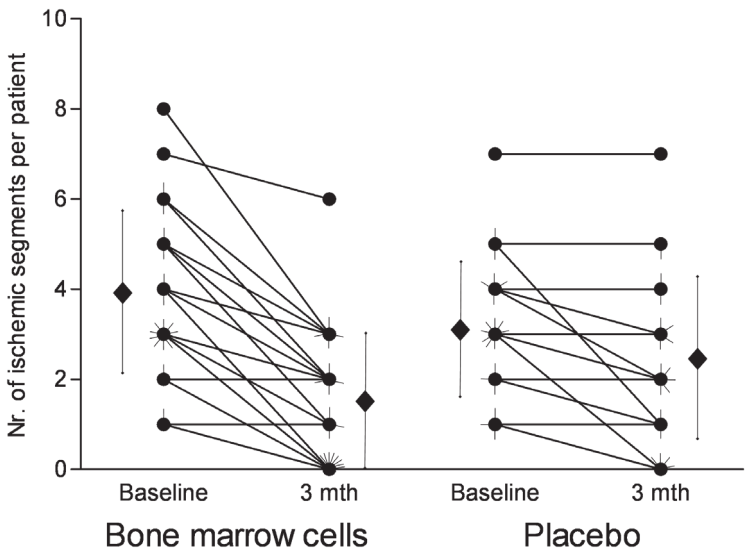

Figure 2
Improvements in segments with inducible myocardial ischemia as assessed by SPECT. For each additional individual with the same value, a spoke is added to the circle data marker. In the bone marrow cell group, there is a larger decrease in segments with inducible ischemia than in the placebo group (P<0.001).

Myocardial perfusion in injected and non-injected segments

In the 25 bone marrow cell-treated patients, a total of 213 injections were targeted at 92 ischemic myocardial segments (3.7±0.6 injected segments per patient). In the 25 placebo-treated patients, a total of 207 injections targeting 97 ischemic myocardial segments were performed (3.9±0.5 injected segments per patient, P=0.38).

In the bone marrow cell group, 52/92 injected segments (57%) increased at least 1 point in rest or stress perfusion (Figure 3). In these patients, 15/333 non-injected segments (5%) showed an improved perfusion (P<0.001). In the placebo group, an improved perfusion was observed in 19/97 injected segments (20%) and in 19/328 non-injected segments (6%)(P<0.001). The percentage of injected segments with an improved perfusion was significantly higher in the bone marrow cell group than in the placebo group (57% vs. 20%, P<0.001). The percentage of non-injected segments with an improved perfusion was similar in both groups (5% vs. 6%, P=0.48).

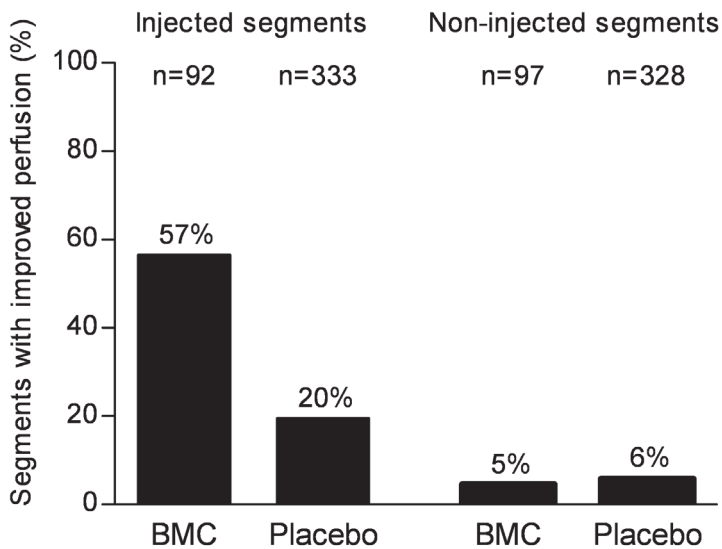

Figure 3

Percentage of injected and non-injected segments showing improved perfusion (defined as an increase of ≥1 point in rest or stress perfusion) in both groups. The percentage of injected segments with improved perfusion was higher in the bone marrow cell (BMC) group than in the placebo group (57% vs. 20%, P<0.001). For non-injected segments, the percentage of improved segments was not significantly different between the bone marrow cell group and the placebo group (5% vs. 6%, P=0.48).

LV function and volumes

Paired MRI was performed in 22 bone marrow cell-treated patients and in 18 placebo-treated patients. In this subset of patients, no differences in baseline characteristics existed between the groups. At baseline, LV volumes and LVEF were not significantly different between the 2 groups (Table 2). In the bone marrow cell group, LVEF increased from 56±12% to 59±11% (P=0.019, figure 4). In the placebo group, LVEF was 54±10% at baseline and 53±10% at 3 months (P=0.55). When the 2 groups were compared, the absolute increase in LVEF was significantly larger in bone marrow cell-treated patients (change +3%, 95% CI 0.5, 4.7 vs. -1%, 95% CI -2.1, 1.1, P=0.027). In both treatment groups, no significant changes in LV end-diastolic volume and LV end-systolic volume were noted (Table 2).

Table 2. LV function and volumes as assessed by MRI

	Bone marrow cell group (n=22)	Placebo group (n=18)	P-value
LVEF (%)			
Baseline	56±12	54±10	0.54
3 months	59±11	53±10	0.11
Change from baseline	+3±5	-1±3	0.027
Change from baseline, P-value	0.019	0.55	
LV stroke volume (ml)			
Baseline	98±47	94±16	0.51
3 months	103±20	92±17	0.063
Change from baseline	+5±10	-2±11	0.048
Change from baseline, P-value	0.030	0.45	
LVESV (ml)			
Baseline	83±47	87±41	0.77
3 months	79±45	86±40	0.61
Change from baseline	-4±15	-1±10	0.45
Change from baseline, P-value	0.092	0.64	
LVEDV (ml)			
Baseline	181±54	181±50	1.00
3 months	182±54	178±49	0.80
Change from baseline	+1±15	-3±19	0.43
Change from baseline, P-value	0.76	0.43	

LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; LVEDV = left ventricular end-diastolic volume

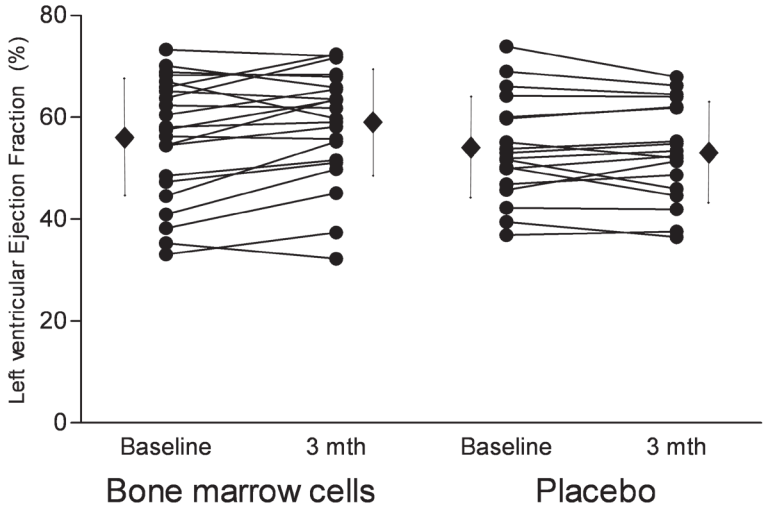

Figure 4
Individual changes in LV ejection fraction. In the bone marrow cell group, LVEF increased significantly (P=0.019), whereas LV ejection fraction remained unchanged in the placebo group (P=0.55). Improvement in LV ejection fraction was significantly greater in the bone marrow cell group (P=0.027).

Clinical outcome

Complete clinical follow-up data were available in 24 patients in the bone marrow cell group and 25 patients in the placebo group. Assessment of the clinical status according to the CCS class revealed a significant improvement in the bone marrow cell group from 3.0±0.6 to 2.3±0.7 at 3 months, and 2.2±0.6 at 6 months (absolute difference at 6 months, -0.79, 95% CI -1.10, -0.48, P<0.001, Figure 5A). In the placebo group, no significant difference in CCS class was observed (2.9±0.7 vs. 2.6±0.8 vs. 2.5±0.9, absolute difference at 6 months -0.39, 95% CI -0.9, 0.12, P=0.058). In line with this observation, quality-of-life increased from 56±9% to 64±12% at 3 months and 69±12% at 6 months (absolute improvement at 6 months 13.0%, 95% CI 8.2, 17.9) in bone marrow cell-treated patients (P<0.001, Figure 5B). In the placebo group, a modest but significant improvement in quality-of-life was noted (57±11% vs. 61±14% vs. 64±17%, absolute improvement at 6 months 6.3%, 95% CI 0.5, 13.0, P=0.036). The improvement in CCS score and quality-of-life was significantly greater in the bone marrow cell group (P=0.032 and P=0.037 respectively).

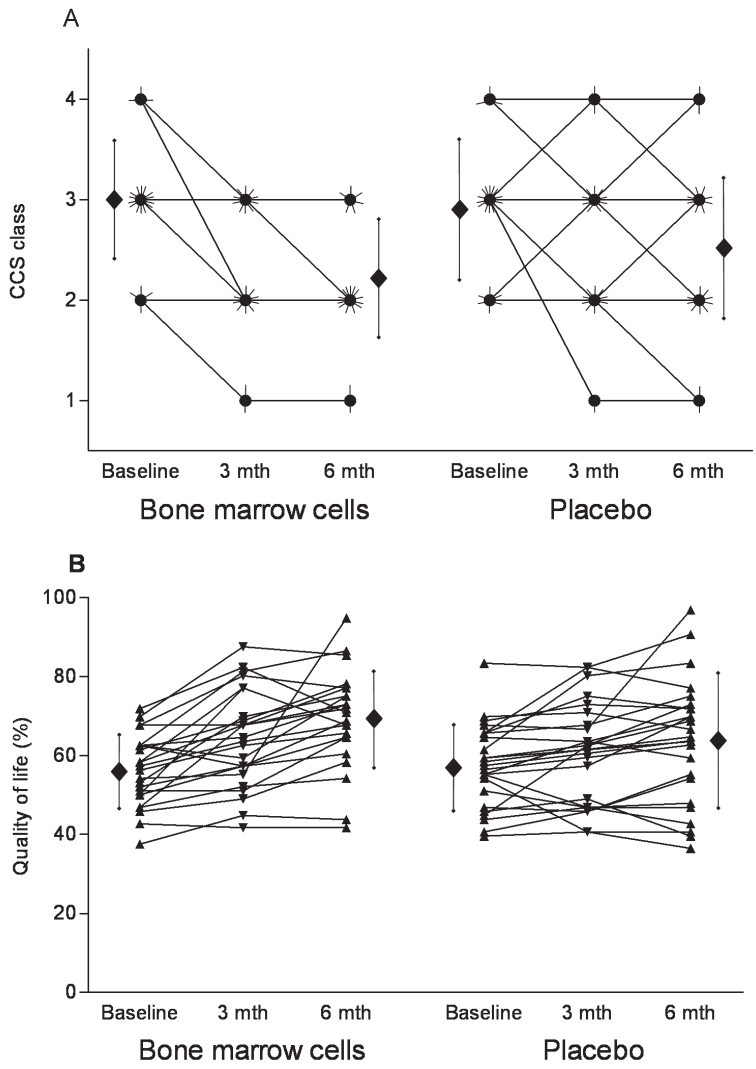

Figure 5
(A) CCS class and (B) Quality-of-life at baseline and at 3 and 6 months follow-up in both groups. For CCS class (A), a spoke is added to the circle data marker for each additional individual with the same value. CCS class improved significantly in the bone marrow cell group (P<0.001), whereas no significant improvement was observed in the placebo group (P0.058). Improvement in CCS class was significantly greater in the bone marrow cell group (P=0.032) Quality-of-life improved significantly in both groups (P<0.001 in the bone marrow cell group and P=0.036 in the placebo group). Quality-of-life showed a larger improvement in the bone marrow cell group (P=0.037).

Exercise capacity

Bicycle exercise testing at all time points was available in 24 bone marrow cell-treated patients and 25 placebo-treated patients. In the bone marrow cell group, the maximal workload achieved increased from 107±29 Watt to 114±34 Watt at 3 months and 116±32 Watt at 6 months (P=0.012). In the placebo group, no significant change in maximal workload was detected (101±35 Watt vs. 99±34 Watt vs. 103±41 Watt, P=0.49). The time to significant ST-segment depression increased from 7.0±1.7 minutes to 8.3±1.7 minutes at 3 months and 8.1±2.0 minutes at 6 months in bone marrow cell-treated patients (P=0.001). No significant change in time to significant ST-segment depression was observed in placebo-treated patients (8.5±3.0 minutes vs. 8.6±3.2 minutes vs. 8.1±2.6 minutes, P=0.25). The change in time to significant ST-segment depression improved more in the bone marrow cell group than in the placebo group (treatment effect 1.3 minutes, 95% CI 0.6, 2.0, P=0.001).

Safety data

During the 6 months follow-up period, no arrhythmias were observed in any of the patients. In addition, electrocardiograms at rest, on 24 hour Holter recordings and during exercise testing revealed no arrhythmias in both study groups.

In the bone marrow cell group, 1 patient died 2.5 months after the injection procedure because of myocardial ischemia leading to acute heart failure. At 3.5 months, another bone marrow cell-treated patient developed a relevant stenosis in an arterial bypass graft which was located outside the injected myocardial territory. This patient underwent a percutaneous coronary intervention and completed the study.

In the placebo group, >0.5 cm pericardial effusion was detected on 2D-echocardiography in an asymptomatic patient 2 days after the injection procedure and pericardiocentesis was subsequently performed. One cerebrovascular accident was observed in another placebo-treated patient with a history of transient ischemic attacks. Four months after the injection procedure, one placebo-treated patient was diagnosed with spondylodiscitis, caused by a staphylococcus aureus infection. Furthermore, one patient in the placebo group was diagnosed with breast cancer at 5.5 months follow-up.

DISCUSSION

The current randomized, double-blind, placebo-controlled trial investigates the effect of intramyocardial bone marrow cell injection on myocardial perfusion and LV function in patients with chronic ischemia who are ineligible for conventional treatment. The main finding of the present trial is that bone marrow cell injection was associated with an improved myocardial perfusion and an increased LVEF. A more pronounced improvement in CCS score, quality-of-life and exercise capacity was observed in bone marrow cell-treated patients as compared to placebo-treated patients.

Animal model studies suggest that bone marrow cell therapy for ischemic heart disease may improve myocardial perfusion and contractile performance.[15] These observations may be related to differentiation of bone marrow cells in endothelial cells, smooth muscle cells or cardiac myocytes. In addition, the bone marrow cells may secrete paracrine factors that promote angiogenesis, exert cytoprotective effects, recruit resident cardiac stem cells or alter mechanical properties of myocardial scar tissue. These pre-clinical findings provided the rationale for the initiation of various clinical studies investigating bone marrow cell therapy as a novel treatment for ischemic heart disease. In most clinical studies, bone marrow cells were infused in the infarct-related artery few days after percutaneous coronary intervention for acute myocardial infarction.[1-3]

Only limited data are available in patients with severe angina and chronic ischemia ineligible for conventional revascularization. Initially, 4 non-randomized pilot studies (with a total of 64 patients) reported that intramyocardial bone marrow cell injection in chronic ischemic myocardium is safe and feasible.[12,16-18] Also, bone marrow cell injection seemed to be associated with a beneficial effect on angina, myocardial perfusion and LV function. A placebo effect, however, may have attributed to these beneficial effects since these studies did not comprise a control group.

Recently, 2 small-sized randomized-controlled trials were performed in patients with chronic ischemia refractory to medical therapy.[6,7] Losordo et al. reported the feasibility and safety of intramyocardial injection of GCSF-mobilized CD34+ stem cells.[7] There was no significant difference in angina frequency, exercise time, or CCS score, but this may have been due to underpowering for these outcomes. In the PROTECT-CAD trial, the effect of intramyocardial bone marrow cell injection on clinical outcome, myocardial perfusion and LV function was assessed in 19 bone marrow cell-treated patients and 9 placebo-treated patients.[6] Bone marrow cell injection was associated with a modest

increase in exercise capacity and LVEF compared with placebo. However, no difference in myocardial perfusion between bone marrow cell-injected patients and placebo-treated patients was observed.

In the present trial, bone marrow cell injection resulted in a significant improvement in anginal symptoms, quality-of-life and exercise capacity, in line with the PROTECT-CAD. Also, the currently observed increase in LVEF in bone marrow cell-treated patients is consistent with the modest increase in LVEF in PROTECT-CAD. The present trial, however, is the first randomized, double-blind, placebo-controlled trial to report that the improvement in anginal symptoms and LV function was accompanied by a significant improvement in myocardial perfusion. Particularly, the decrease in the number of ischemic myocardial segments per patient was significantly greater in bone marrow cell-treated patients as compared to placebo-treated patients, implicating a more pronounced improvement in myocardial perfusion as observed in the PROTECT-CAD trial. This may be related to the 2-fold higher number of bone marrow cells administered in the current trial ($98\pm7x10^6$ cells vs. up to $42\pm28x10^6$ in PROTECT-CAD). Although this specific issue has not yet been addressed in large clinical studies, a recent trial in acute infarction patients suggested the possibility of a dose-response relationship.[19]

In the past decades, a number of therapies has emerged for the management of refractory angina pectoris. For example, treatment with the metabolic agent Ranolazine increased exercise time with 23.9 seconds compared to placebo treatment[20]. However, the time to onset of ST-segment depression during exercise testing did not change and the effect on myocardial perfusion was not investigated. Another adjunctive therapy, enhanced external counterpulsation (EECP), was associated with a decrease in angina pectoris frequency, but no significant effect on exercise time was observed[21]. Importantly, the effect of EECP on myocardial perfusion has not been investigated in a randomized trial. An invasive technique that has been investigated for the treatment of angina pectoris is transmyocardial laser revascularization. Although improvement in anginal complaints was reported in the initial studies[22], a large randomized, blinded trial did not show a beneficial effect of laser revascularization when compared to a sham procedure[23]. In particular, no improvement in myocardial perfusion was observed after Ranolazine administration, EECP or after tramsmyocardial laser revascularization in randomized studies.

In the present trial, the beneficial effect of bone marrow cell injection on CCS class, quality-of-life and exercise capacity may be classified as modest. However, in contrast

with the aforementioned techniques, the beneficial effect on clinical parameters was accompanied by objective improvements in myocardial perfusion and LV function.
In the current trial, small but significant improvements in quality-of-life and myocardial perfusion were observed in the placebo group. These improvements may be related to improved adherence to use of medication, and life style changes (diet changes, smoking cessation, physical activity). Finally, a beneficial effect of the intramyocardial placebo injections on myocardial perfusion, possibly by inflammation-mediated stimulation of angiogenesis, cannot be ruled out.
The present trial had several limitations. The trial was not designed to assess whether the improvement in myocardial perfusion and global LV function is associated with reduced mortality and morbidity; moreover, whether the improvements noted after 3 and 6 months follow-up are sustained over time needs further study. Finally, the results of the current study do not provide information on bone marrow cell homing, retention and survival, and do therefore not explain the cellular mechanisms responsible for the observed improvement in myocardial perfusion.
In summary, the results of the current randomized, double-blind, placebo-controlled trial demonstrate that intramyocardial bone marrow cell injection in patients with chronic ischemia is associated with significant improvements in anginal symptoms, myocardial perfusion and LV function.

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